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Review

Human natural killer cells and other innate lymphoid cells in cancer: Friends or foes?

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ABSTRACT

Innate lymphoid cells (ILC) including NK cells (cytotoxic) and the recently identified "helper" ILC1, ILC2 and ILC3, play an important role in innate defenses against pathogens. Notably, they mirror analogous T cell subsets, regarding the pattern of cytokine produced, while the timing of their intervention is few hours *vs* days required for T cell-mediated adaptive responses. On the other hand, the effectiveness of ILC in anti-tumor defenses is controversial. The relevance of NK cells in the control of tumor growth and metastasis has been well documented and they have been exploited in the therapy of high risk leukemia in the haploidentical hematopoietic stem cell transplantation setting. In contrast, the actual involvement of helper ILCs remains contradictory. Thus, while certain functional capabilities of ILC1 and ILC3 may favor anti-tumor responses, other functions could rather favor tumor growth, neo-angiogenesis, epithelial-mesenchymal transition and metastasis. In addition, ILC2, by secreting type-2 cytokines, are thought to induce a prevalent pro-tumorigenic effect. Finally, the function of both NK cells and helper ILCs may be inhibited by the tumor microenvironment, thus adding further complexity to the interplay between ILC and tumors.

1. Introduction

Natural Killer cells play an important role in innate defenses against cancer and viral infections. Their constitutive storage of perforin and granzymes as well as the rapid production of IFN-γ and TNF-α upon cell triggering, allows their prompt intervention against tumor cells and favors the initiation of inflammation [1–5]. NK cells are regulated by an array of receptors mediating either inhibitory or activating signals that finely tune their function [6–8]. In humans, important inhibitory receptors are those specific for HLA-class I (HLA-I) molecules and are represented primarily by killer Ig-like receptors (KIR) and CD94/NKG2A. While KIR recognize allotypic determinants of classical HLA-I molecules, CD94/NKG2A receptor recognizes the non-classical HLA-E molecules [9]. Among the numerous activating NK receptors, a central role for tumor or leukemia cell recognition and killing is played by NKp46, NKp30 and NKp44 (collectively referred to as natural cytotoxicity receptors, NCR), NKG2D and DNAM-1 [10]. Other surface molecules, including 2B4, NTBA and NKp80, appear to function primarily as co-receptors [11–13]. Notably, also activating forms of KIR have been documented [6]. For some of them the specificity for HLA-I alleles has also been demonstrated [14], but their effective role in regulating target cell recognition remains poorly clarified. The other activating NK receptors generally recognize on target cells ligands that are up-regulated or expressed *de novo* upon cell stress, tumor transformation or viral infection. While stressed healthy cells are "protected" by their surface HLA-I molecules interacting with KIR or CD94/NKG2A, tumor or virus-infected cells may lose HLA-I expression becoming susceptible to the NK-mediated attack [7].

As revealed by studies performed during the last decade, NK cells belong to a new family of innate lymphoid cells (ILC). NK cells and the

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other ILC derive from a $CD34^+$ common lymphoid progenitor and lack receptors encoded by rearranging genes [15]. Five major groups of lymphoid cells have been identified on the basis of the cytokines produced and the transcription factors necessary for their development. While NK cells represent the "cytotoxic ILC" the others have been defined "helper ILC" because they are non-cytolytic and produce sets of cytokines unique for each subset. Thus, ILC1 secrete IFN-γ, ILC2 type-2 cytokines including IL-5, IL-13 and small amount of IL-4, NCR^+ILC3 secrete primarily IL-22 while NCR^-ILC3 (or LTi-like) produce IL-17 and TNF-α [16,17]. Remarkably, the helper ILC mirror corresponding T helper subsets secreting the same sets of cytokines. As a consequence, these cytokines shape both innate and adaptive responses, although with different timing and dynamics. Moreover, cytokines produced either by NK cells and ILC1 (primarily IFN-γ) or by ILC2 (type 2 cytokines), influence the downstream specific responses by favoring T lymphocyte polarization towards Th1 or Th2, respectively [18].

As mentioned above, NK cells and the other ILC derive from $CD34^+$ common lymphoid precursors that reside in the BM. However, precursors of ILC have been detected in tissues such as tonsils [19], liver and decidua [20–27]. Moreover, the source of $CD34^+$ precursors used for hematopoietic stem cell transplantation (HSCT) influences the prevalent type of progeny (mainly NK cells vs ILC3) [28]. In addition, drugs, including steroids [29,30] or tyrosin kynase inhibitors (Damele, Frontiers in Immunology 2018, in press) may also cause a differential recovery of NK cells and/or ILC3 *in vitro*, suggesting the possibility of preferential growth of one or another subset *in vivo*. A remarkable characteristic of ILC is their plasticity, *i.e.* their ability to convert into one another as a consequence of signals delivered by the tissue microenvironment, cytokines or pathogens [16].

While the potential anti-tumor and anti-leukemia activity of NK cells has been well documented, the actual role of other ILC is controversial (see below). In addition, the particular microenvironment established at the tumor site, may exert a complex inhibitory effect on NK cell phenotype and function and, possibly, on ILC.

2. Effect of the tumor microenvironment on NK cell function

It is generally accepted that NK cells can eliminate tumors particularly at initial stages and also play an important role against tumor spread and metastases [31,32]. On the other hand, the anti-tumor activity of NK cells maybe greatly compromised in established solid tumors in terms of cell numbers and efficacy [33]. It is conceivable that NK-mediated tumor cell targeting may reflect substantial differences between tumor cells from small initial lesions and established solid tumors or metastases [34]. Indeed, the surface expression of HLA-I molecules and of various ligands for activating receptors may be downregulated or upregulated rendering different tumor cells more or less susceptible to NK cell-mediated recognition and killing. However, what features do have in common initial-stage and metastatic cells? Although, they are quite distant in the process of tumor progression, they are both relatively accessible to NK cells as they are not yet included in an organized niche harboring and protecting cancer cells [35]. Indeed, the presence of such a protective niche and the establishment of a tumor microenvironment are responsible not only for the poor accessibility of different effector cells with anti-tumor potential but also for their functional inactivation. As illustrated in previous contributions [35,36], the tumor microenvironment contains an assortment of different non-malignant cell types, including mesenchymal stromal cells, endothelial cells, fibroblasts, macrophages and other myeloid cells, as well as soluble factors and cytokines with inhibitory activity released from tumor cells themselves and from non-malignant tumor-associated (TA) cells [37–42]. These factors include the IDO-induced kynurenin, prostaglandin E2 (PGE2), TGF-β, IL-10 [43–45] (Fig. 1). An effect common to some of these soluble factors is the downregulation of activating NK receptors. Thus, not only NK cells have to face a barrier protecting tumor cells, but are also "disarmed" and fail to recognize and kill tumor cells. Of note, hypoxia, typical of the microenvironment of different tumors, has a similar effect, causing downregulation of activating receptor with the remarkable exception of CD16 [46]. Since the lytic machinery of NK cells is not substantially compromised, mechanisms involving CD16 engagement, such as antibody dependent cell-mediated cytolytic activity (ADCC), are preserved [46]. However, it should be also considered that the transcriptome analysis of NK cells exposed to hypoxia revealed that some of their functional capabilities, including the ability to migrate in response to specific stimuli may be substantially modified (Parodi M. et al. Frontiers in Immunology, 2018 in press) (Fig. 1).

NK-mediated tumor cell killing *in vitro* requires appropriate ratios between NK effector and tumor target cells. However, such ratios may not be reached, particularly in established primary or metastatic tumors. In this context, when suboptimal NK/melanoma cell ratios were tested *in vitro*, a paradoxical phenomenon was observed. Thus, after an initial NK-mediated killing of tumor cells, melanoma cells became resistant to lysis. This resistance reflected the increased surface expression of HLA-I molecules and a partial downregulation of NKG2D ligands. Both effects were consequent to IFN-γ production by NK cells. Of note, the analysis of melanoma specimens revealed a higher HLA-I expression on tumor cells proximal to NK cell infiltrates [47]. These data suggest that, in some instances, NK cells may favor mechanisms of tumor escape. Remarkably, resistance to NK cell-mediated killing *in vitro* could be partially overcome by activating NK cells with IL-15 [46]. On the other hand, NK cells present in tumor-infiltrated lymph nodes of melanoma patients may display a potent cytolytic activity against autologous tumor cells, suggesting that the tumor microenvironment may also generate/recruit a peculiar subset of highly cytolytic NK cells [48].

Another potentially harmful effect exerted by NK cells has recently been described [49]. This refers to the epithelial-mesenchymal transition (EMT), an important mechanism that favors the metastatic dissemination of different tumor types. In a similar context, NK cells have been shown to increase the aggressiveness of melanoma cells favoring their switch from proliferative to invasive forms by inducing upregulation of stemless or EMT typical markers. These effects, similar to those induced by EMT-promoting cytokines, result in morphological changes of tumor cells, inhibition of proliferation and increased invading capacity (Fig. 1). These changes are dependent on the engagement of activating NK receptors and the release of IFN-γ and TNF-α. EMT induction was also accompanied by the upregulation of protective HLA-I expression on tumor cells and by the downregulation of activating NK receptors, two effects that greatly compromise the NK-mediated tumor cell killing. These data further indicate that NK cells, despite their strong cytolytic potential against tumors, may be responsible of a paradoxical effect favoring tumor escape and even inducing changes that may lead to tumor invasion and to the initial stages of metastatic dissemination (Fig. 1). Remarkably, however, the overall effect of NK cells in tumor metastasis is protective, as revealed by different studies [50]. Thus, although, in some instances, NK cells could favor mechanisms of tumor escape, these cells still represent a potentially reliable immunotherapeutic tool that may be significantly improved by the choice of appropriate priming cytokines or the combined use of tumor specific antibodies to exploit the NK-mediated ADCC function [33,51].

3. PD1 expression in NK cells

The inhibitory receptor PD1 functions as an important checkpoint in the immune responses. Originally discovered by Honjo in T cells [52], it was found to downregulate excessive T cell responses, thus contributing to the homeostasis of the immune system. However, it became evident that tumor cells may induce the expression of PD1 in T cells, accompanied by the upregulation of PD1-ligands (PD-L1 and PD-L2) on their own surface. Thus, while under physiologic conditions PD1 functions as a brake to avoid unwanted responses, including auto-reactivity, in cancer it may contribute to the block of anti-tumor responses, upon

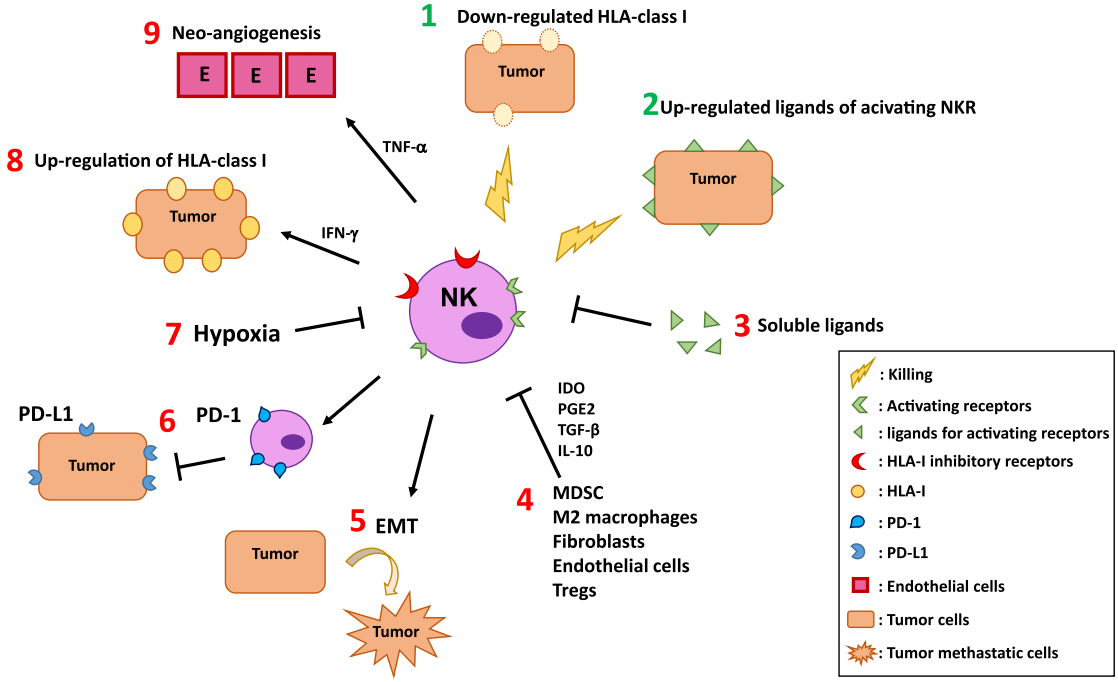

Fig. 1. The ambiguous functional activities of human NK cells in the tumor microenvironment.
Although NK cells play an overall positive role in defenses against tumors, their function can be drastically impaired in the microenvironment of established solid tumors. Thus, while NK cells can kill tumor cells that have downregulated the surface expression of HLA-I surface molecules (a mechanism of tumor escape from T cell-mediated control) **(1)** and can sense tumor cells that have upregulated or expressed *de novo* different ligands recognized by their activating receptors **(2)**, a number of mechanisms that impair their function may take place in the tumor microenvironment. First, the ligand themselves, if released in soluble form from tumors may inhibit NK cell function by functioning as "decoy" ligands **(3).** A number of cells attracted and/or instructed by tumor cells, such as Tregs, myeloid-derived suppressor cells (MDSC), M2-polarized macrophages release factors or cytokines that compromise the anti-tumor effect of NK cells (for example, by downregulating the expression of activating NK receptors) **(4)**. In some instances, even functionally important cytokines released by NK cells, such as IFN-γ and TNF-α, induce a paradoxical effect. For example, IFN-γ can induce the upregulation on tumor cells of HLA-I molecules. HLA-I interact with KIR and NKG2A, inhibitory NK receptors, and block NK cytotoxicity **(8)**. IFN-γ may also induce the epithelial-mesenchymal transition (EMT), a first step of metastatic spread **(5)** which is also favored by the TNF-α-induced neoangiogenesis **(9)**. The hypoxia, frequently characterizing established tumor lesions, impairs NK cell function by downregulating the activating NK receptors **(7)**. Another particularly important mechanism is the expression of PD1 inhibitory receptor induced, in mature NK cells, by the tumor microenvironment. When tumors express PD1 ligands (primarily PDL1), the PD1-PD-L1 interaction may lead to a major impairment of NK cell function. This can be restored by anti-PD1 or anti-PD-L1 mAbs, that disrupt the PD1-PD-L1 inhibitory interaction **(6)**. These findings have important implications in the therapy of HLA-I deficient tumors which are not susceptible to T cell-mediated control.

interaction with PD-L1 (or PD-L2) –positive tumor cells. It has recently been shown that also NK cells can express PD1, particularly those associated to tumors [53]. In this study, PD1 was first detected on small proportions of NK cells from some healthy donors. Interestingly, all individuals with PD1$^+$ NK cells in their peripheral blood (PB) were seropositive for cytomegalovirus (CMV). Analysis of patients with ovarian carcinoma revealed that most patients had PD1$^+$ NK cells in their PB. More importantly, the proportions of such cells were much higher in the ascitic fluid, strongly suggesting that the tumor environment may be responsible for the *de novo* expression of PD1 on NK cells. Of note, all PD1$^+$ cells were confined to the most mature CD56^{dim} CD16$^+$ NK cells. These findings further reinforce the importance of therapies based on anti-PD1 (or PD-L1) blocking mAbs to disrupt the inhibitory interaction. Indeed, many metastatic carcinomas lose the expression of HLA-I molecule, thus becoming undetectable by T cells. However, this escape mechanism renders tumor cells susceptible to NK-mediated killing. Thus, anti-PD1-based therapies may restore responses to PD-L1$^+$ tumors independent on their HLA-I surface expression levels. In this context, determination of PD-L1 expression in tumors became a major issue to define the eligibility for treatment with anti-PD1 or anti-PD-L1 mAbs. These evaluations are mostly based on bioptic specimens. Since these may provide only a partial information on the positive/negative PD-L1 expression of given tumors, studies have been focused to determine the number of biopsies required to more closely reflect the PD-L1 expression status on whole tumor sections [54,55]. It is evident that a correct information is extremely important to decide on the eligibility of a given patient to treatment.

4. Anti-leukemia activity of NK cells in haemopoietic stem cell transplantation (HSCT)

HSCT is the life-saving therapy for high risk acute leukemias, *i.e.* leukemia either relapsing or responding poorly to chemotherapy or with adverse cytogenetic/molecular features. Unfortunately, only for two thirds of patients in need of HSCT a suitable HLA-compatible donor can be found, while for the remaining patients no alternative effective therapeutic option is available. In an attempt to rescue these patients, in the late 90's a new transplantation approach has been developed, namely the HLA-haploidentical HSCT (haplo-HSCT). It is also of note that, in the case of HSCT from a matched unrelated donor, the average time from the diagnosis to transplantation is 2 months. As a consequence, a high percentage of patients (up to 50%) succumb before HSCT, due to their disease or infections. In contrast, in most haplo-HSCT, a donor (a parent or a sibling) is promptly available. A crucial graft manipulation in this highly HLA-incompatible transplant (at least in its original setting), is the extensive T lymphocyte depletion, to avoid the occurrence of severe GvHD. In haplo-HSCT, in the absence of T cells, NK cells play a major role in the anti-leukemia effect. This effect is, at least in part, associated to the NK-alloreactivity, due to a KIR-HLA mismatch in the donor vs recipient direction. Notably, the size of the alloreactive NK subset was found to directly correlate with a more efficient anti-leukemia effect and with the survival probability [56–58].

This T-depleted haplo-HSCT offers also the chance of monitoring *in vivo* the NK cell development from $CD34^+$HSC [59]. Similar to *in vitro* models of NK cell differentiation, the first NK cell population detectable in PB of HSCT-recipient is CD94/$NKG2A^+$, while KIR^+ NK cells appear in the PB after 6–8 weeks. In order to further filling the gap between HSCT and the appearance of mature, KIR^+ alloreactive NK cells derived from donor $CD34^+$ HSC, a novel transplantation setting has been developed [60,61]. This is based on the selective depletion of $TCR\alpha\beta^+$ T cells (responsible of GvHD) and B cells, so that the infused mononuclear cells, in addition to $CD34^+$ cells, also contain mature alloreactive NK cells and $TCR\gamma\delta^+$ T cells, both endowed with anti-leukemia activity [62,63]. Thus, the prompt availability of leukemia-reactive effector cells has further improved the clinical outcome of patients with otherwise fatal leukemia, particularly in the case of AML for which the haplo-HSCT using pure $CD34^+$ could save only one third of patients. Thus, from ~30% and 60% overall survival probability for AML and ALL, respectively, the percentages of patients cured with the new approach was ~70% for both ALL and AML [61]. Taken together, these data indicate that NK cells exert a strong anti-leukemia activity, at least in the haplo-HSCT setting. This may be favored by the relatively low number of blasts remaining after conditioning and their distribution in tissues. Thus, although some IL-1-releasing AML blasts could affect NK cell differentiation *in vitro* [64], it is likely that residual leukemia cells in post-transplant patients cannot establish a suppressive tumor microenvironment. Remarkably, the ability of $CD34^+$ precursors to give rise also to ILC3, including the NCR^-ILC3 with LTi-like activity [28], may favor mucosal tissue repair and the organization of lymphoid aggregates and secondary lymphoid organs, contributing to the reconstitution of the adaptive immune system, impaired by chemo/radiotherapy during the conditioning regimen.

5. The controversial role of ILC1, ILC2, and ILC3 in tumors

While NK cells, with the few exceptions illustrated above, display an anti-tumor activity and NK cell defects favor tumor growth (at least in mice), the actual involvement of other ILC in tumor inhibition or promotion is still contradictory [65].

Thus, ILC1, thanks to the production of IFN-γ, may polarize T cells towards Th1 responses and macrophages towards M1, both useful for anti-tumor responses. In addition, ILC1 induce DC maturation and favor tumor antigen presentation through the production of TNF-α, a cytokine that also promotes tumor cell apoptosis. On the other hand, IFN-γ may induce EMT transition and metastasis and TNF-α neoangiogenesis. Although, ILC1 secrete the same cytokines of NK cells, the anti-tumor activity of NK cells is better defined, being related primarily to their cytolytic activity. Along this line, the conversion of effector NK cells into type 1 ILC have been proposed as a tumor escape mechanism in mice [66]. ILC2, in line with type 2 responses, appear to play a prevalent pro-tumorigenic activity. Thus, while the induced eosinophils may play an anti-tumorigenic activity *via* IL-5, ILC2-associated cytokines such as IL-13 and, in part, IL-4 (produced in low amounts by human ILC2) favor the generation of M2 macrophages, myeloid-derived suppressor cells (MDSC), regulatory T cells (Treg) and induce Th2 polarization. Also ILC3 may be responsible of opposite effects on tumor growth [16]. Thus, NCR^-ILC3 induce the generation of tertiary lymphoid structures (TLS) which, in turn, favor T cell infiltration and responses to tumor antigens. Importantly, the frequency of TLS appears to correlate with better prognosis in patients with lung tumors [67]. On the other hand, the production of IL-22 (from NCR^+ILC3) and IL-17 (from NCR^-ILC3) may promote tumor growth. A further complexity in defining the actual role of helper ILC in tumor immunity is related to their plasticity. It is conceivable that ILC may contribute to shape the tumor microenvironment; on the other hand, they may be induced to switch in to one another. For example, IL-1β and IL-12 promote the conversion of ILC2 into IFN-γ-producing ILC1. Moreover, conversion of ILC1 into NCR^+ILC3 can promote tumorigenesis.

6. Concluding remarks

It is now clear that different cell types that belong to the innate immunity, particularly NK cells, play a relevant role in anti-tumor defenses thanks both to their effector function and to their ability to shape the downstream adaptive immunity towards Th1 responses which are favorable for tumor control. On the other hand, they may also play an unwanted role in tumor promotion, particularly under the influence of the tumor microenvironment. Recently, it has been shown in a murine tumor model that NK cells can recruit and interact with conventional type 1 dendritic cells. This interaction was compromized by tumor cells, resulting in impaired NK-mediated anti-tumor activity [68]. It is of note that mechanisms exploited by tumors to subvert immune responses, have a physiological role in certain normal tissue environments. For example, the decidual environment induces NK cell inactivation/polarization, macrophages and other myeloid cell polarization towards a suppressive phenotype and Treg generation [69]. The recent finding that not only T, but also NK cells can express PD1 opened new therapeutic perspectives in the cure of HLA-I-defective tumors (undetectable by T cells) based on the use of mAbs that target PD1 or PD-L1. In addition, harnessing of NK cells can be achieved by blocking KIR or NKG2A, thus overcoming the inhibitory effect consequent to their interaction with tumors or leukemia expressing HLA-I. In this context, in the haplo-HSCT setting, it is possible to render “alloreactive” virtually all donor NK cells.

The plasticity of ILC may be exploited by inducing the preferential generation/expansion of subsets endowed with anti-tumor activity. This goal may be achieved by culturing $CD34^+$ HSC with appropriate cytokine mixtures. The resulting “useful” ILC populations may be infused in new protocols of adoptive cell therapy of cancer and leukemia. However, further investigations are clearly needed not only to find markers that can directly identify the various ILC subsets, but also to assess their homing properties and their ability to traffic toward different tumor sites.

Conflict of interest

The authors declare no conflict of interest.

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